627. *Synthetic Polypeptides. Part I. Synthesis of Oxazolid-2 : 5-diones and a New Reaction of Glycine.*

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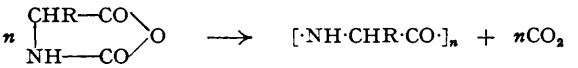
Improved techniques for preparing benzyl chloroformate and oxazolid-2 : 5-diones by the Leuchs–Bergmann method are described. Other 4-substituted oxazolid-2 : 5-diones are prepared by the treatment of the corresponding α-amino-acids with carbonyl chloride. It is shown that glycine reacts with sodium carbonate to form disodium methylamine-1 : *N*-dicarboxylate, which is converted into oxazolid-2 : 5-dione by the action of thionyl chloride or carbonyl chloride.

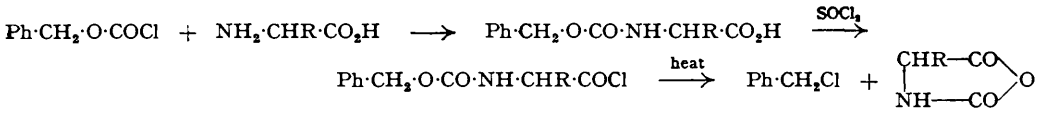
THE adaptation (by Woodward and Schramm, *J. Amer. Chem. Soc.*, 1947, **69**, 1551) of the Leuchs method (*Ber.*, 1906, **39**, 857) for preparing synthetic polypeptides has shed fresh light on the problem of synthesising polypeptides of high molecular weight from the several α-amino-acids. Leuchs's method involves decomposition of the appropriate oxazolid-2 : 5-dione by heat or by compounds such as water and organic bases :



As the polymers were generally insoluble, even if of low molecular weight, the reaction was stopped by precipitation of the product before a high polymer could be formed. By using a soluble copolymer in the appropriate solvent Woodward and Schramm allowed the reaction to proceed to higher molecular weights, and this technique has since been used by other workers (Waley, Watson, and Hanby, *Nature*, 1948, **161**, 132; Astbury *et al.*, *Nature*, 1948, **162**, 596).

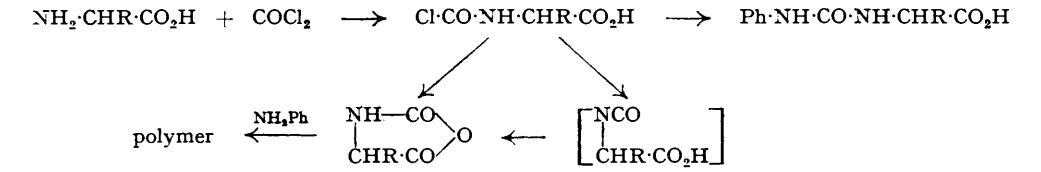
In this paper (Part I) the synthesis of some intermediates is described. Parts II and III (succeeding papers) are concerned with " polymerisation " of the intermediates.

The Leuchs–Bergmann route to oxazolid-2 : 5-diones involves three stages :



Benzyl chloroformate cannot be purified by distillation, and an improved technique for its preparation is provided by treatment of benzyl alcohol with carbonyl chloride directly at low temperatures. The purer benzyl chloroformate obtained was reflected in a purer *N*-carbobenzyloxy-α-amino-acid. In the case of glycine the conversion of the *N*-carbobenzyloxy-derivative into oxazolid-2 : 5-dione was improved by using acetic anhydride as medium for reaction with thionyl chloride and elimination of benzyl chloride, thus reducing the two reactions to a single operation. This technique was preferred to the other methods described for the preparation of oxazolid-2 : 5-dione because of the instability of the product.

Some substituted oxazolid-2 : 5-diones were made directly from the α-amino-acid, in dioxan suspension, by treatment with carbonyl chloride. This reaction is analogous to the preparation of *iso*cyanates from amine salts by carbonyl chloride; it was first used by Fuchs (*Ber.*, 1922, **55**, 2943) and was extended to other α-amino-acids by Baird, Parry, and Robinson (B.P. Appl. 20,406 and 27,401/1947; see also Levy, *Nature*, 1950, **165**, 152; Farthing and Reynolds, *ibid.*,



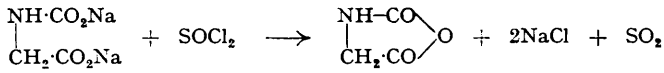
p. 647). The intermediate carbamyl chloride was detected by its reaction with aniline to form, *e.g.*, *N*-phenylhydantoic acid in the case of glycine. This method was preferred for all the α-amino-acids used, except for glycine, with which reaction is slower. It is necessary to use pure α-amino-acid to simplify the purification of the oxazolid-2 : 5-dione, and because it is difficult to establish accurate criteria of purity, on account of instability. The carbonyl chloride reaction is quantitative under the proper conditions, the only possible impurities being the carbamyl chloride, Cl·CO·NH·CHR·COCl, and the derived *iso*cyanate, OCN·CHR·COCl, which are both liquid (Robinson *et al.*, *loc. cit.*). Thus, if pure α-amino acid was used and the product was recrystallised at least twice (which removes all the chlorine) it was assumed that pure oxazolid-2 : 5-dione had been prepared. It was found that several commercial samples of L-leucine were heavily contaminated with methionine (cf. Mueller, *Science*, 1935, **81**, 50) which made the preparation of pure *iso*butyl-L-oxazolid-2 : 5-dione impossible and in earlier qualitative experiments on the " polymerisation " a product of m. p. 65—70° (compared with 76·5—78° for the pure compound) had to be used. For the later detailed work this difficulty was overcome (Part III).

The oxazolid-2 : 5-diones are internal anhydrides of the hypothetical carbamic acid $HO_2C{\cdot}NH{\cdot}CHR{\cdot}CO_2H$. Only derivatives, such as salts, esters, and amides, of carbamic acids are known. Barium methylamine-1 : *N*-dicarboxylate (*N*-carboxyglycine barium salt) has been prepared from glycine, barium hydroxide, and carbon dioxide (Siegfried, *Ber.*, 1906, **39**, 398. *Z. Physiol. Chem.*, 1905, **44**, 85; 1906, **46**, 401; 1908, **54**, 436 : Schryvver *et al.*, *Biochem. J.*, 1921, **15**, 636; 1924, **18**, 1070; Blanchetière, *Compt. rend.*, 1923, **179**, 1629), and a series of basic salts is known (Neuberg and Kerb, *Biochem. Z.*, 1912, **40**, 498). Ester salts have been reported by Frankel *et al.* (*J. Amer. Chem. Soc.*, 1943, **65**, 1671). A possible relation to *in vivo* reactions has been investigated (Siegfried *loc. cit.*; Stadie and O'Brien, *J. Biol. Chem.*, 1936, **112**, 723). It may be that the carbamate salts are involved in carbon dioxide transfer, and the formation of carbamates may be a delicate means of blocking amino-groups *in vivo* for the purposes

of polypeptide synthesis. The existence of sodium salts of these carbamic acids has been demonstrated. Disodium methylamine-1 : *N*-dicarboxylate (*N*-carboxyglycine disodium salt) has been isolated and its structure proved. When a solution of equimolecular amounts of glycine and sodium carbonate in water is treated with methanol this salt separates as a white solid :

$$NH_2{\cdot}CH_2{\cdot}CO_2H + Na_2CO_3 = NH_2{\cdot}CH_2{\cdot}CO_2^- + Na^+ + HCO_3^- = {}^-O_2C{\cdot}NH{\cdot}CH_2{\cdot}CO_2^- + 2Na^+$$

Whatever the value of the equilbrium constants these equilibria must be present and the disodium salt must be the product least soluble in aqueous methanol. Analyses are correct for this formula and not for a mechanical mixture of glycine and sodium carbonate. The *X*-ray diffraction pattern of the powder is different from that of such a mechanical mixture. A mechanical mixture reacts very slowly with thionyl chloride, whereas this product reacts spontaneously with evolution of heat and with charring. By reaction with thionyl chloride or carbonyl chloride in dioxan or ethyl acetate, oxazolid-2 : 5-dione is formed in 32% yield. With carbonyl chloride, this reaction is ambiguous, but the reaction with thionyl chloride shows that a carbon atom is attached to the nitrogen :



The disodium salt is soluble in aqueous barium hydroxide and so does not form carbonate ions on dissolution. On cooling the known barium salt separates. In the case of the barium salt the equilibrium must lie entirely in favour of the dicarboxylate, or of hydrogen carbonate because it is water-soluble, and only on heating does the irreversible precipitation of barium carbonate occur.

This new synthesis of oxazolid-2 : 5-dione could not be extended to substituted derivatives. Sodium salts were prepared from DL-alanine, α-amino*iso*butyric acid, L-leucine, and DL-phenylalanine, but these could not be converted into the 4-substituted oxazolid-2 : 5-diones. The synthesis of oxazolid-2 : 5-dione has the merit of not requiring carbonyl chloride; but if carbonyl chloride can be used, the benzyl chloroformate route, as described above, is preferable.

Experimental.

Oxazolid-2 : 5-diones are extremely water-sensitive. All apparatus was oven-dried at 110° for at least 1 hour. Solvents were dried by conventional methods and stored over " Drierite " (anhydrous calcium sulphate). Thionyl chloride was purified by fractional distillation from quinoline and then from raw linseed oil. A convenient qualitative test for oxazolid-2 : 5-diones was to cover a trace with water (faint effervescence), and then add a small drop of aniline (violent effervescence, finely divided precipitate). The precipitate of polypeptide was then confirmed by the biuret reaction. Immediately after isolation the oxazolid-2 : 5-diones were dried over phosphoric oxide and flaked petroleum wax *in vacuo* at room temperature, and then stored *in vacuo* over phosphoric oxide. The storing desiccator was opened for the shortest possible time when samples were required. All exits from apparatus to atmosphere were protected by drying tubes containing " Drierite " or magnesium perchlorate.

Benzyl Chloroformate.—Preparation according to Bergmann, using a toluene solution of carbonyl chloride (*Ber.*, 1932, **65**, 1194), gave coloured crude products. In an improved method carbonyl chloride was passed into redistilled benzyl alcohol (282 g.) with stirring and cooling with ethanol–solid carbon dioxide, at such a rate that the internal temperature remained at −20° to −30°. After 4 hours the internal temperature fell. No more carbonyl chloride was passed in and the reaction mixture was left to attain room temperature. Compressed air, dried by passage through concentrated sulphuric acid and then over flaked sodium hydroxide, was passed through the gas lead, with stirring, for 24 hours. The stirrer and gas lead were removed and the flask was evacuated at the water-pump for 5 minutes. The faintly cloudy liquid was filtered and a trace of less dense oil separated. There was obtained a clear colourless liquid (418 g., 91%) which during 1 year assumed a pale straw colour. It was powerfully lachrymatory and traces on apparatus, etc., were destroyed rapidly with ethanolic ammonia. In a larger-scale preparation more of the by-product was obtained (1 g. from 985 g. of benzyl alcohol); it had b. p. 108° but was not identified.

N-*Carbobenzyloxyglycine.*—The method of Bergmann (*loc. cit.*) was used and refined. After addition of benzyl chloroformate to glycine in 4N-sodium hydroxide the reaction mixture was extracted with ether, and the ethereal layer rejected. The aqueous layer was stirred with " Suma " carbon and filtered. The filtrates and washings were cooled, with stirring, in ice. Concentrated hydrochloric acid was added dropwise with stirring until the mixture was acid to Congo-red. White shining plates separated (cf. Bergmann, *loc. cit.*, who reports that an oil separates and solidifies). The solid was collected, washed with water, and dried in air at 80° (170 g., 85%). Recrystallisation from chloroform–light petroleum gave white needles, m. p. 121° (127·5 g., 63·8%).

Oxazolid-2 : 5-dione.—(*a*) *From* N-*carbobenzyloxyglycine.* Traces of oxazolid-2 : 5-dione were obtained by heating *N*-carbobenzyloxyglycine (10 g.) in acetyl chloride (25 c.c.) and acetic acid (5 c.c.) under reflux for 1 hour. None was obtained by boiling the ester with acetic anhydride. The method of Bergmann (*loc. cit.*) was simplified by using acetic anhydride as a medium. *N*-Carbobenzyloxyglycine (15 g.), acetic anhydride (15 c.c.), and thionyl chloride (7·0 c.c.) were warmed gently to dissolve the solid. On cooling, no precipitate was formed. The solution was boiled for 5 seconds and cooled.

Oxazolid-2 : 5-dione which separated was collected, washed with ether, and dried (4·2 g.). The liquors were treated with light petroleum (b. p. 60—80°), to yield more product. (Total yield of crude product : 7·2 g., 96%.)

(*b*) *From glycine and carbonyl chloride* (method of Robinson *et al.*, *loc. cit.*). Finely ground glycine (8 g.) was stirred with dioxan (375 c.c.) in a 2-l. three-necked flask, fitted with a gas lead, water reflux-condenser, and liquid-sealed stirrer and immersed in a bath at 40°. Carbonyl chloride was slowly passed in for 4—5 hours, the glycine slowly dissolving. Dry air was blown through the solution overnight at room temperature. The stirrer and gas lead were removed, the condenser changed to the downward position, and the dioxan removed at the water pump with the bath at 40°. A liquid remained, and on further heating at 40° *in vacuo* solidified; the solid was washed out with ether. If the oil did not solidify after 1 hour's further heating it crystallised on addition of ether. The white crystals were dried (9·2 g., 85%).

In a similar reaction the dioxan was partly removed at 20° at the water-pump during 10 hours. There was left a pale yellow solution smelling of dioxan and an acid chloride, but not of carbonyl chloride. The solution reacted exothermically with dry benzyl and methyl alcohols with formation of hydrogen chloride, and gave a black tar with pyridine. To a portion in chloroform was added dry aniline; heat was evolved and a solid precipitated. Next day this was collected and crystallised from alcohol; it had m. p. 195°, alone or mixed with *N*-phenylhydantoic acid (from phenyl *iso*cyanate and aqueous glycine). The acid chloride was therefore *N*-chloroformylglycine.

(*c*) *From disodium methylamine*-1 : N-*dicarboxylate*. Carbonyl chloride (20 g.) was dissolved in ethyl acetate (317 g.) cooled in ice in a 2-l. three-necked flask fitted as in (*b*). Disodium methylamine-1 : *N*-dicarboxylate (*N*-carboxyglycine disodium salt) (36 g.) was rapidly added. The mixture effervesced and the stirrer was started. After 3 minutes the effervescence stopped and carbonyl chloride was passed in for 5 minutes. The ice-bath was removed. After 30 minutes the reaction mixture was heated to the b. p. during 15 minutes. The mixture was filtered hot and the filtrate made up to 500 c.c. with light petroleum. Oxazolid-2 : 5-dione was filtered off, washed with light petroleum, and dried (6·0 g., 27%).

Disodium methylamine-1 : *N*-dicarboxylate (10 g.) and ethyl acetate (150 c.c.) were stirred at room temperature, and thionyl chloride (5·0 c.c., a slight excess) was added. When the reaction had finished and the orange mixture had cooled to room temperature, ethyl acetate (100 c.c.) was added and the mixture refluxed for 10 minutes and filtered hot. The filtrates were concentrated *in vacuo* to *ca.* 100 c.c. Light petroleum (100 c.c.) was added, the solution cooled, and oxazolid-2 : 5-dione filtered off (2·0 g., 32%).

When dioxan was used as solvent similar results were obtained. Use of acetyl chloride in place of thionyl chloride or carbonyl chloride gave no oxazolid-2 : 5-dione.

Properties. For " polymerisations " the crude product was recrystallised from ethyl acetate, and if stored for more than a few days was again recrystallised before use and dried over-night (cf. Mark *et al.*, *J. Appl. Physics*, 1949, **20**, 531). It was recrystallised from freshly distilled acetic acid but slight decomposition to polymer occurred; if the acetic acid had been boiled with a trace of acetic anhydride no decomposition occurred. The dione is more soluble in polar than in non-polar solvents and may be also recrystallised from adiponitrile, dimethylformamide, and methyl methylacrylate. It is insoluble in cold benzene, chloroform, or light petroleum. It is difficult to establish rigorous criteria of purity because of its instability. A stock of *N*-carbobenzyloxyglycine was made and portions rapidly converted into oxazolid-2 : 5-dione as required.

4-*Methyl*-DL-*oxazolid*-2 : 5-*dione*.—(*a*) *From* N-*carbobenzyloxy*-DL-*alanine* (*m. p.* 114°). This derivative was prepared similarly to *N*-carbobenzyloxyglycine. The solid acid was dissolved in excess of thionyl chloride at room temperature to form the acyl chloride. Excess of thionyl chloride was removed at 40° at the water-pump. The residue was a pale brown oil which lost benzyl chloride when further heated *in vacuo* at 40°. The resulting pale brown oil could not be crystallised or purified. It gave the reactions of an unstable oxazolid-2 : 5-dione. By using the acetic anhydride–thionyl chloride method described for glycine an apparently identical product was obtained.

(*b*) *From* DL-*alanine*. DL-Alanine, finely ground (10 g.), in dioxan (250 c.c.) was treated with carbonyl chloride at 38—40° for 5½ hours, all dissolving. Dioxan was removed at 40°/20 mm., to yield a pale brown oil apparently identical with that obtained under (*a*) above.

4 : 4-*Dimethyloxazolid*-2 : 5-*dione*.—α-Amino*iso*butyric acid was prepared from purified acetone by the Strecker reaction (*Org. Synth.*, Coll. Vol. II, p. 69). The amino-acid (15 g.) in dioxan (400 c.c.) was treated with carbonyl chloride at 50° (bath-temp.) for 9 hours. After air-blowing and removal of dioxan as before an oil remained. This completely solidified when heated for 1 hour at 40°/20 mm. The product was taken up in a minimum of hot chloroform, the solution was filtered, and 3 volumes of light petroleum were added slowly. White shining crystals separated (15 g., 80%), m. p. 95—97°.

4-*Benzyl*-DL-*oxazolid*-2 : 5-*dione*.—DL-Phenylalanine (*Org. Synth.*, 1941, **21**, 99) (10 g.) was treated with carbonyl chloride in dioxan (200 c.c.) for 2 hours at 40° (bath-temp.). The solution was air-blown and dioxan removed at 40° at the water-pump. The oily product rapidly crystallised and was recrystallised from ethyl acetate–light petroleum, to yield white plates (6·5 g., 60%), m. p. 127° (decomp.).

Attempted Preparation of 4-2′-*Carboxyethyl*-L-*oxazolid*-2 : 5-*dione*.—L-Glutamic acid (10 g.) in dioxan (200 c.c.) was treated with carbonyl chloride at 40° for 10 hours. No change was apparent. After a further 15 hours at 50° the solid dissolved. Dioxan was removed at 40°/20 mm., and the liquid residue heated for 1 hour at 40°/20 mm. The liquid was taken up in ether and precipitated with light petroleum. The precipitated oil was worked with ethyl acetate; pale brown crystals separated and were filtered off. The solid was extremely deliquescent and could not be purified. With water it effervesced rapidly and formed a brown solution which was too dark for application of the biuret test.

4-2′-*Methylthioethyl*-DL-*oxazolid*-2 : 5-*dione*.—DL-Methionine (5 g.) in dioxan (170 c.c.) completely dissolved during treatment with carbonyl chloride at 40° for 10 minutes. Treatment was contined for a further 2·5 hours, the solution air-blown, and dioxan removed at 40° at the water-pump. The residual brown oil did not crystallise when further heated at 40°/20 mm. It behaved as a typical oxazolid-2 : 5-dione in its reaction with water and bases.

4-iso*Butyl*-L-*oxazolid*-2 : 5-*dione*.—L-Leucine commercially available in this country was too crude for conversion into the pure crystalline oxazolid-2 : 5-dione (Found : C, 52·35; H, 9·2; N, 9·35; S, 2·4; SO_4, 0. $C_6H_{13}O_2N$ requires C, 55·0; H, 9·95; N, 10·65%. S, equiv. to 11·2% methionine). This crude product was partly purified through the sodium carbonate complex. L-Leucine (50 g.) and anhydrous sodium carbonate (40·4 g., 1 mol.) in hot water (150 c.c.) were treated with carbon and the solution filtered hot. The filtrate was cooled and next morning the solid was collected, washed with a little methanol, and dried (14 g.). The solid was stirred with water (100 c.c.), and concentrated hydrochloric acid (5·0 c.c.) added to give pH 3. The mixture was boiled and filtered. The filtrates on cooling gave L-leucine (2·8 g.) (Found : C, 53·2; H, 9·5; N, 10·1; S, 0·6%, corresponding to 2·95% of methionine). The residue was recrystallised from water (120 c.c.), to give L-leucine (2·0 g.) (Found : C, 54·6; H, 9·8; N, 9·7; S, 0·28%, corresponding to 1·3% of methionine). Further products recovered from liquors contained 4·3 and 4·4% of sulphur (equivalent to 20% of methionine). Dissolution in aqueous ammonia, and treatment with active carbon, followed by precipitation with acid, only slightly reduced the sulphur content.

The following reaction with carbonyl chloride is typical. L-Leucine (10 g.) in dioxan (330 c.c.) was treated with carbonyl chloride at 40°, and dissolved in 2·75 hours. Carbonyl chloride and dioxan were removed, yielding a brown oil which did not crystallise after a further 90 minutes at 40° at the water-pump. The oil gave the characteristic oxazolid-2 : 5-dione reactions. The purchased samples of L-leucine, as such, could not be converted into a crystalline oxazolid-2 : 5-dione. Samples purified through the sodium carbonate complex were crystallised with difficulty from ether–light petroleum. No other suitable solvent (mixture) could be found. This oxazolid-2 : 5-dione was the most unstable one prepared during this work. Traces of acetic anhydride added to the solvents helped to stabilise it. Usually the compound separated as an oil on addition of light petroleum to the ethereal solution, and the oil crystallised when seeded and shaken violently. The highest m. p. obtained was 65—70° (Woodward and Schramm, *loc. cit.*, give 76·5—78°), after several crystallisations. A sample of L-leucine was eventually obtained which gave a faint sulphur reaction on sodium fusion, but quantitative microanalysis showed unmeasurable traces. This sample proved as difficult to convert into the oxazolid-2 : 5-dione as the previous purified samples, and was eventually shown to contain tyrosine (cf. Coleman, Part III).

Preparation of Disodium Methylamine-1 : N-*dicarboxylate* (N-*Carboxyglycine Disodium Salt*).—Anhydrous sodium carbonate (106 g., 1 mol.) and glycine (75 g., 1 mol.) were dissolved in water (500 c.c.) and the solution filtered. Methanol (1800 c.c.) was slowly and continuously run into the solution during 1 hour, with stirring. A precipitate appeared after 400 c.c. had been added. The white, finely divided *salt* was filtered off, washed with methanol and then with ether, and dried at 100° (92 g., 56·5%). By using less water (450 c.c.) and more methanol (2 l.) the yield was increased (123 g., 75·5%). The product could not be recrystallised [Found : C, 22·45; H, 2·2; N, 8·5; Na_2CO_3 (combustion residue) 64·1. $C_3H_3O_4NNa_2$ requires C, 22·1; H, 1·8; N, 8·6; Na_2CO_3, 65·05. $C_3H_5O_5NNa_2$ (mixture of glycine and Na_2CO_3) requires C, 19·9; H, 2·8; N, 7·7; Na_2CO_3, 58·6%]. Samples were dried at 80° *in vacuo* before analysis. The compound does not lose water on heating until decomposition takes place.

The salt was free-flowing, soluble only in water, and insoluble in inert organic solvents. It was precipitated from aqueous solution only by methanol. Ethanol and acetone were merely salted out from the aqueous phase. Acetic acid and/or acetic anhydride dissolved the salt with effervescence and formation of sodium acetate; the fate of the glycine residue was not investigated. In the absence of organic solvents thionyl chloride reacted violently to yield a black solid from which no organic compound could be isolated. With thionyl chloride in organic solvents oxazolid-2 : 5-dione was formed (see above). The *X*-ray diffraction pattern of the powder was quite different from that of a mechanical mixture of glycine (0·5 mol.) and Na_2CO_3 (0·5 mol.) ball-milled together for 24 hours. With thionyl chloride this mechanical mixture slowly effervesced but did not otherwise change.

The disodium salt (1·0 g.) was added to an excess of saturated aqueous barium hydroxide (20 c.c.). It all dissolved on shaking, to yield a faintly cloudy solution. Cooling and scratching with a glass rod produced fine crystals, which were collected, washed with acetone and then ether, and dried (1·1 g., 100%). The barium salt obtained was identical with that prepared by Siegfried (*loc. cit.*). Boiling the aqueous solution precipitated barium carbonate. The solution gave carbon dioxide on acidification and a positive ninhydrin reaction. The barium salt could not be converted into oxazolid-2 : 5-dione.

Other Sodium Salts.—Similar compounds were similarly prepared from DL-alanine and α-amino*iso*-butyric acid. They resembled in appearance and solubility the compound from glycine. Neither could be converted into the corresponding oxazolid-2 : 5-diones and were not characterised. The less soluble L-leucine and DL-phenylalanine gave less soluble compounds with sodium carbonate. They were also less stable, changing in composition on recrystallisation from water. They could not be converted into the corresponding oxazolid-2 : 5-diones and were not investigated. The derivative from L-leucine was regenerated with acid to yield purer L-leucine (see above).

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